Summary: Pilot Feasibility Study of NSC.CD + 5-FC

3 dose levels assessed; 15 patients treated

- ✔ **Safety of HB1.F3.CD NSCs (no grade 3 or 4 toxicities related to the NSCs)**
- ✔ **Proof of Concept – conversion of prodrug to active drug by NSCs**
- ✔ **No NSC immunogenicity on first exposure**
- ✔ **Fate of NSCs (2 brain autopsies):**
  - **NSCs detected at tumor sites distant from injection site and in contralateral hemisphere at tumor foci**
  - **No evidence of secondary tumors**
- ✔ **Imaging of NSC Distribution via Feraheme-labeling**

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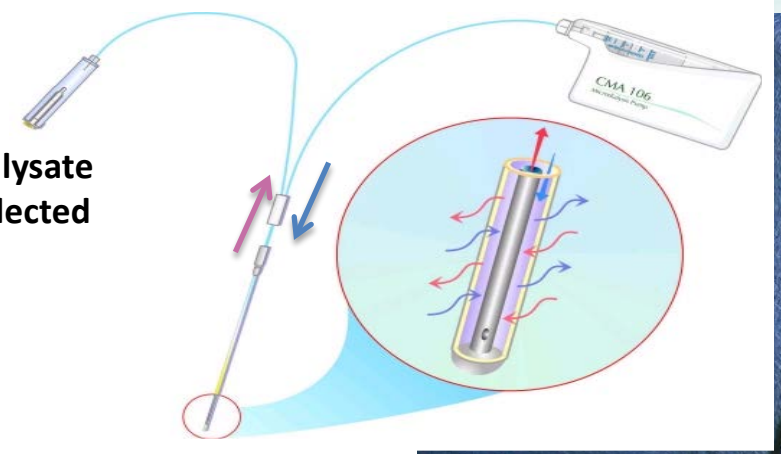
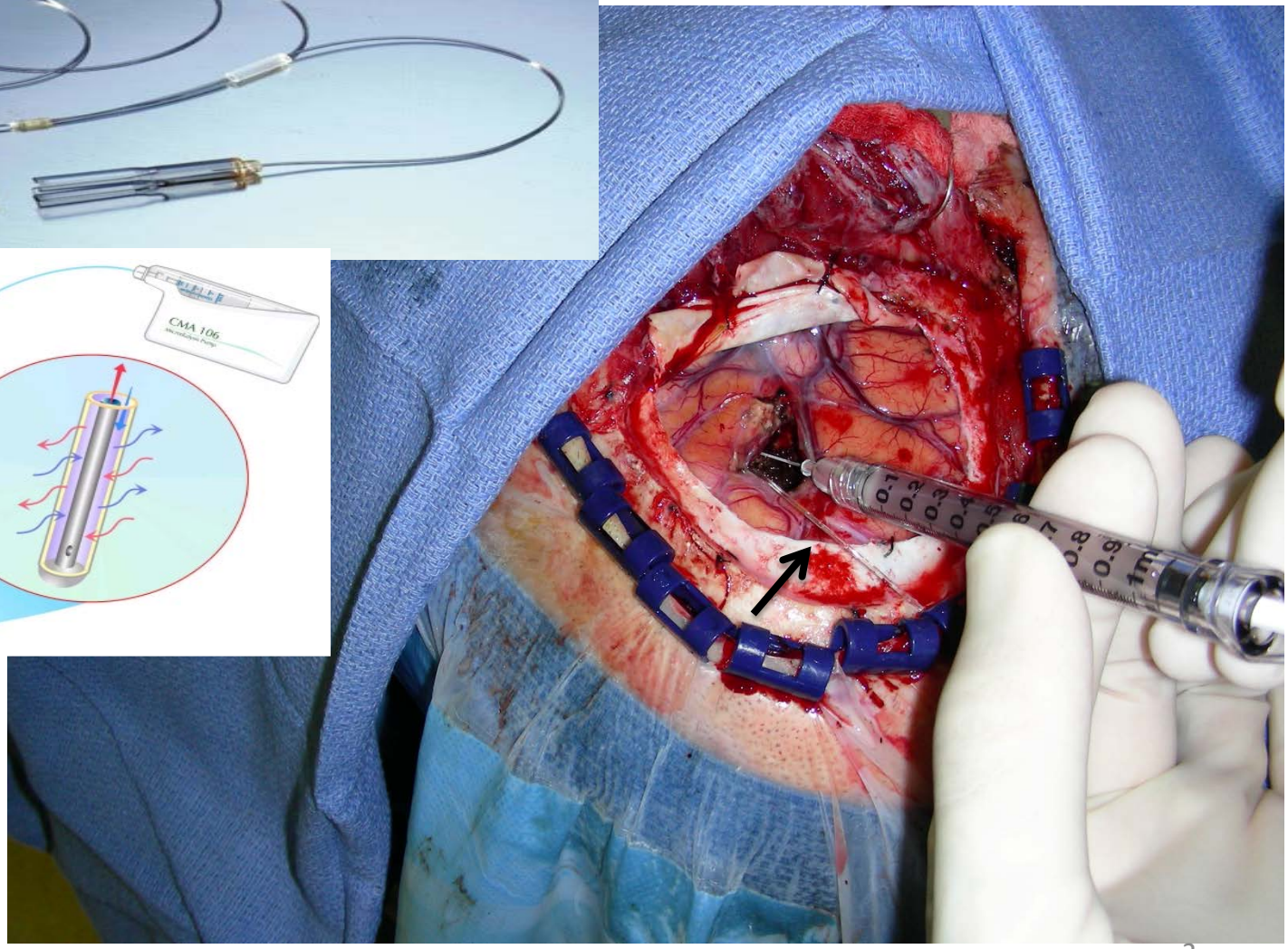
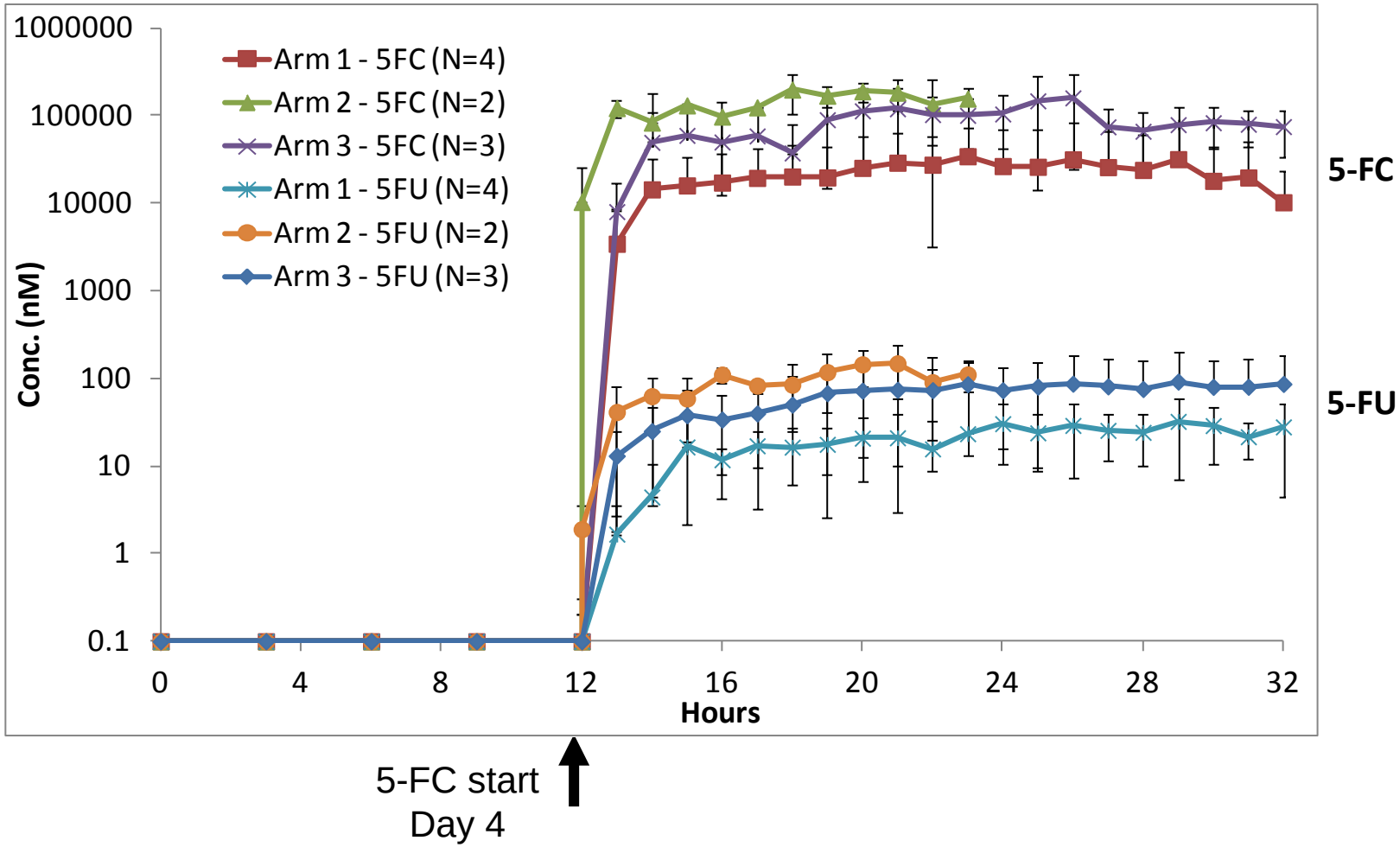
Microdialysis Brain Catheter





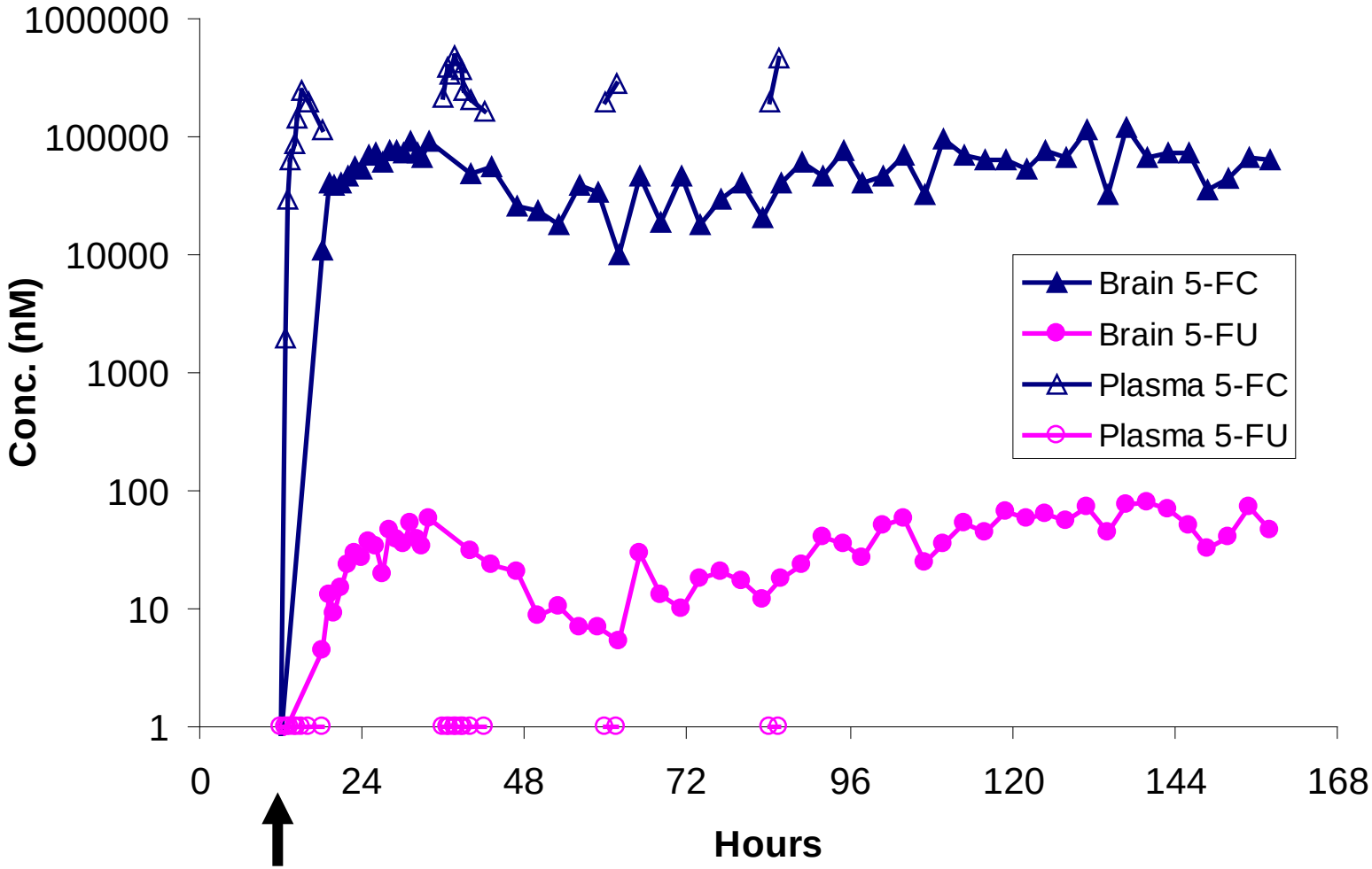
Proof of Concept: CD-NSCs Convert 5-FC to 5-FU in the Brain

Final Intracerebral Microdialysis Data From the Pilot-Feasibility Study



Proof of Concept Using Intracerebral Microdialysis

Pt #4 5-FC & 5-FU Levels in Brain and Plasma



5-FC start

Continuous 5-FU production in brain during 7 days of 5-FC
No 5-FU detected in patient's plasma

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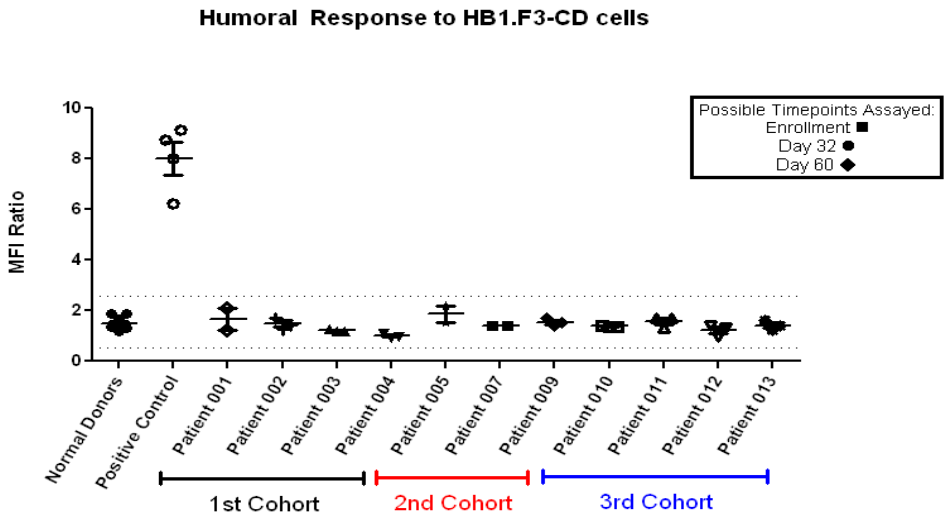
Summary to Date of Correlative Immunologic Studies

Evaluation of Humoral Responses to NSCs

Pt serum samples obtained prior to surgery and on days 32 and 60 underwent evaluation by flow cytometry to assess for antibody binding to NSCs.



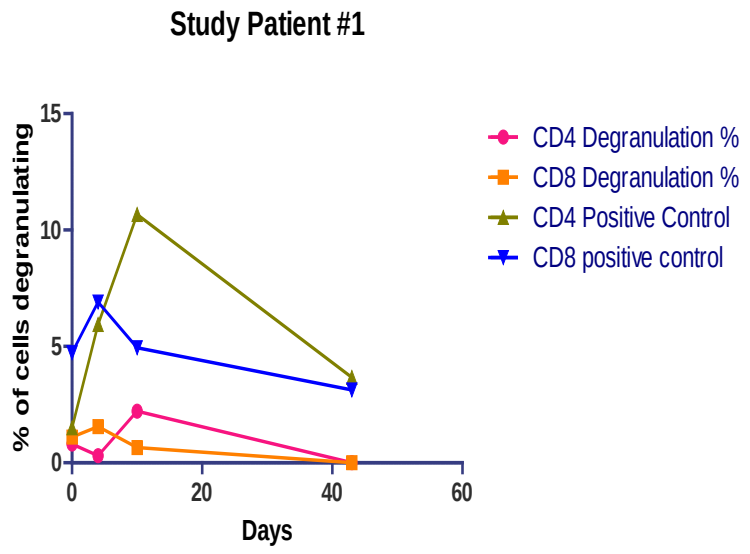
Each point for the patient sera represent a mean of triplicate assays.
The dotted lines indicate a "normal MFI" range of 0.55 to 2.59 that represents a 95% prediction interval based on 2.37 SD in each direction (Hahn and Meeker, *Statistical Intervals* Willey and Sons, 1991).
The 'positive control' is serum sample from a patient that exhibited a positive response to HLA-A1 antibodies. The patient was dropped from the study as a result.

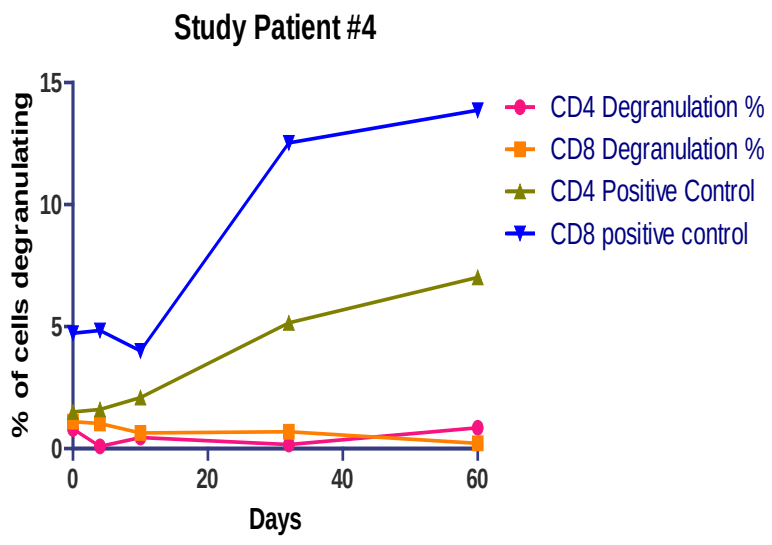
No detection of anti-NSC antibodies outside the normal range after first exposure to NSCs.

Summary to Date of Correlative Immunologic Studies

Evaluation of T-cell Responses to NSCs

Pt PBMCs collected from baseline, d4, d10, d32 and d60 and evaluated by co-culture with NSCs followed by flow cytometry evaluation of degranulation (CD107 mobilization).





No evidence of elevated levels of degranulating CD4+ or CD8+ T-cells

Summary to Date of Correlative Immunologic Studies

Evaluation of the peripheral presence of NSCs

Quantitative PCR assay using v-*myc* primers performed in pt. PBMCs prior to surgery, and days 4, 10, 32, and 60.

All patient samples are negative for detection of peripheral persistence of v-*myc* (NSCs)

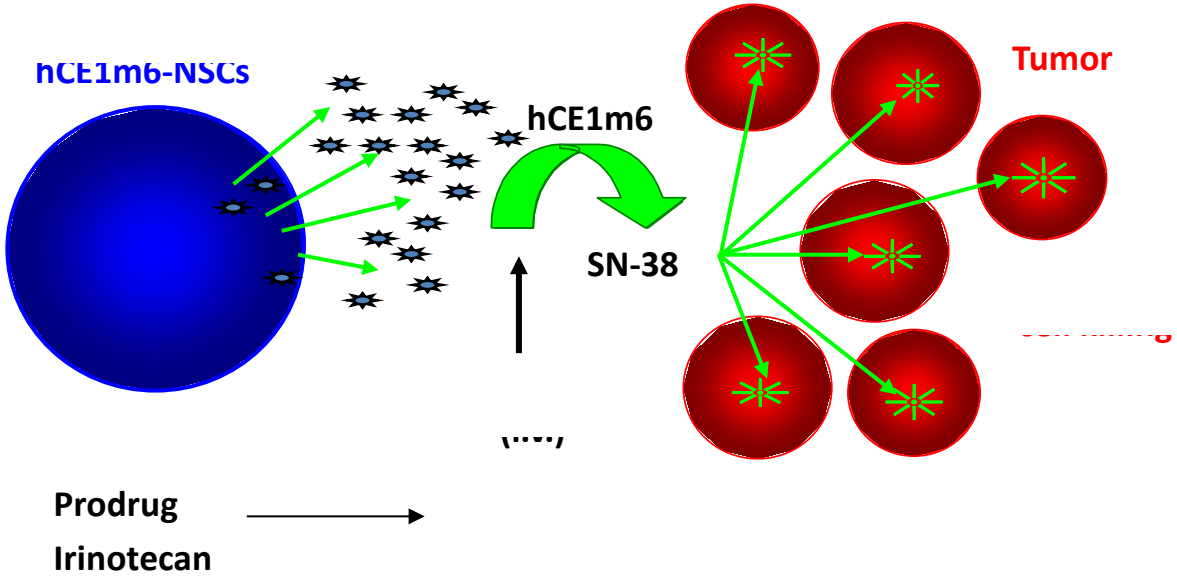
Evaluation of replication competent retrovirus (RCR)

Patient DNA from whole blood analyzed by PCR for RCR-specific sequences prior to surgery, at 3 months, 6 months, 1 year after study tx, and then annually.

RCR testing is negative on all patient samples to date for up to 1 year out.

Proposed Phase I Study of hCE1m6-NSCs and Irinotecan

A Multi-Center Phase I Study of Intracranially Administered CE-Expressing NSCs in Combination with Intravenous Irinotecan for the Treatment of Recurrent High-Grade Gliomas



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Dose Escalation Schema

Dose Level	Initial Enrollment	If 1 DLT, Addt'l Enrollment	NSC Dose	Irinotecan 125 mg/m^2/week (administered 2 days post-NSCs) Week					
				1	2	3	4	5	6
-1	3	3	2.5×10^7	X					
1	3	3	5×10^7	X					
2	3	3	5×10^7	X		X			
3	3	3	5×10^7	X	X	X			
4	3	3	5×10^7	X	X	X	X		
5	3	3	1×10^8	X	X	X	X		
6	3	3	1.5×10^8	X	X	X	X		
MTD Cohort 2	16		MTD	X	X	X	X		

Estimated sample size: 24 for dose escalation + 16 for MTD cohort 2 = 40 patients

Phase I Study: Primary Objectives

To define the recommended phase II doses of intracranially administered hCE1m6-NSCs in combination with intravenous irinotecan.

Based on the maximum-tolerated doses (MTDs) and toxicity profile in patients with recurrent high-grade gliomas.

To determine the biologic activity of hCE1m6-NSCs by comparing SN-38 concentrations in the brain after treatment with hCE1m6-NSCs and irinotecan compared to irinotecan alone.

Determine the Biologic Activity of the hCE1m6-NSCs

Intracerebral microdialysis will be performed in:

1) **The first 6 study patients to measure SN-38 levels in brain when treated with the lowest dose of NSCs (50 million)**

To compare to data collected from MTD cohort 2 patients and assess if treatment with higher doses of NSCs results in more SN-38 in the brain.

2) **A cohort of 16 patients with recurrent glioblastoma (MTD cohort 2) receiving the MTD of study treatment**

- while receiving hCE1m6-NSCs + irinotecan (n=8) **or**
- while receiving irinotecan alone (n=8)

To determine if the addition of NSCs to irinotecan increases concentrations of SN-38 in the brain.

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Secondary Objectives

- To assess for possible development of NSC immunogenicity after first exposure and with repeat doses of NSCs.
- To evaluate the intracerebral distribution of NSCs by using iron-labeling as a cellular tracker.
- To describe the clinical benefit (defined as stable disease, partial response, or complete response) in patients who receive treatment with repeat cycles of NSCs and irinotecan.
- To determine, at time of autopsy, the fate of the NSCs.

Immunologic Correlative Studies

Blood samples drawn:

prior to surgery
on day 1 of weeks 3 and 5 during cycle 1
on day 1 of week 3 with subsequent treatment cycles

To assess for possible development of anti-NSC antibody and T cell responses

If patients develop increasing anti-NSC antibody and/or T cell responses:

We will further investigate the immunogenic component by specifically assessing the collected blood samples for:

anti-hCE1m6 antibody and T-cell responses
anti-adenoviral antibody and T-cell responses

Development of a Possible Clinical Immune Response to the NSCs

Aliquots of serum will be saved from baseline blood samples drawn for immunologic correlative studies prior to the start of study treatment.

If a patient were to develop signs/symptoms of a possible immune response to the NSCs, these stored serum samples along with newly collected ones would be analyzed via Luminex cytokine panels.

Findings consistent with the presence of a "cytokine storm" would add strength to the conclusion that the patient is experiencing an immune reaction to the NSCs.

Management of a Possible Clinical Immune Response to the NSCs

Start treatment with dexamethasone or increase the dose if a patient is already on dexamethasone.

Based on the severity of clinical symptoms and the presence of supporting correlative cytokine data, a decision will be made about whether to stop the patient's study treatment or not.

If a patient develops a grade 2 adverse event suspected to be due to an immune response to the NSCs, dexamethasone will be started or increased, and if the adverse event improves to ≤ grade 1, the patient will be allowed to continue study treatment.

If a patient develops **a grade 3 or 4 adverse event that is at least possibly related to development of an immune response to the NSCs**, in addition to treatment with dexamethasone, study treatment will be permanently discontinued.

Assessment of NSCs in Systemic Circulation

Blood samples drawn:

prior to surgery
on day 1 of weeks 3 and 5 during cycle 1
on day 1 of week 3 with subsequent treatment cycles

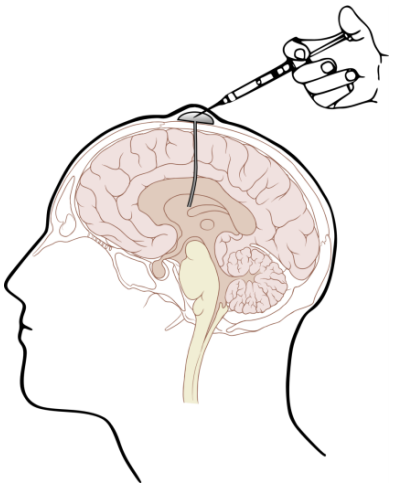
To look for the presence of NSCs in the systemic circulation.

Do not anticipate any increase in systemic toxicity if hCE1m6 were to get into systemic circulation, since the amount of CE from the NSCs would be negligible Compared to the total quantity of CE present in the liver and gut.

For confirmation, will collect PK data from patients who will first receive irinotecan alone one week before treatment with NSCs and irinotecan and compare systemic levels of SN-38.

Repeat Doses of NSCs

Starting with dose level 2, patients will receive repeat doses of NSCs via a Rickham catheter/reservoir system placed at the time of surgery.



The City of Hope Brain Tumor Program has experience with using Rickhams to repeatedly administer genetically –modified T-cells during a 2 week period to glioma patients.

Feasibility experiment to assess binding and viability of HB1.F3.CD NSCs when administered through a Rickham Catheter:

- **100% recovery of NSCs through the Rickham after flushing**
- **NSC viability did not decrease for at least 6 hours after thawing and administering through a Rickham.**

Currently performing this experiment with hCE1m6-NSCs

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Informed Consent

Reviewer: Provide an explanation of fetal tissue and how we obtained the fetal tissue

"The neural stem cells used in this study come from human fetal tissue. These cells were derived from the brain of a 15 week old fetus and were donated for research use. Neural stem cells are immature cells that have not yet developed into different types of brain cells. Neural stem cells have a natural ability to distribute themselves within a tumor and find other sites of tumor in the brain…."

Reviewer: The Rickham catheter needs to be more fully described and provide a picture in the consent form.

Agree that a picture of the Rickham catheter would be very helpful, one will be added to the consent form.

"If the Rickham catheter were to become infected, it would be removed, and you would be treated with antibiotics, otherwise, the Rickham catheter will remain in your brain after you finish study treatment."

Informed Consent

Reviewer's concerns about the patient's ability to withdraw from the study once study treatment has started

"Long-Term Follow Up: The long-term health benefits and risks of receiving genetically-modified neural stem cells are not well understood, and the fate of the neural stem cells following administration is not known. Therefore, we will continue to follow you indefinitely after you have finished the treatment phase of this study or if you decide to withdraw from the study at any time."

Reviewer's concerns about the consenting process

When possible, a research subjects advocate will be present during the consenting session.

PIs at other sites will receive training on how to consent study patients.
A checklist of points to cover during the consenting session will be provided to and completed by the site PIs.

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